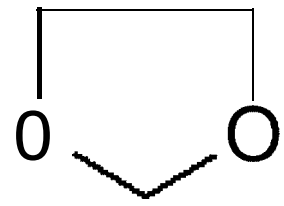
AR201-13084A



1,3-DIOXOLANE

CAS Number 646-06-o

USEPA HPV Challenge Program Submission

20 November 2000
Revised: 12 June 2001

Submitted by:
Dioxolane Manufacturers' Consortium
Members
Ferro Corporation
Ticona

Prepared by:
Toxicology and Regulatory Affairs
One Rowlands Road
Flemington NJ 08822
908-806-7068

TABLE OF CONTENTS

Executive Overview

1,3-Dioxolane (Dioxolane) is a stable reaction product of ethylene glycol and formaldehyde used primarily as a monomer for production of high-molecular weight polyacetals. Other uses are as a chemical intermediate, process solvent and stabilizer for halogenated solvents. It is a volatile liquid that is miscible with water in all proportions. Dioxolane has a production volume exceeding one million pounds per annum; hence, it was listed by the Environmental Protection Agency on its list of chemicals in the High Production Volume Chemical Challenge Program. This document is Industry's response to the EPA challenge to define the toxicologically relevant data set within the HPV guidelines and fill gaps in our knowledge.

The physicochemical properties of Dioxolane are well defined. High water solubility and moderate volatility are defining characteristics. Environmental fate data are available from a combination of experimental and modeling data. Dioxolane is not readily biodegradable, is stable in water for over at year in the pH 4 to 9 range and has an estimated photodegradation half-life in air in the range of 10 to 30 hours. Predicted values for fugacity have been calculated with the MacKay model, which indicate an initial primary distribution in water: however, experience shows that in open systems it rapidly volatilizes to air where it will be destroyed by photooxidation. Fish, daphnia and green algae are only acutely affected by Dioxolane at concentration levels greater than several hundred ppm. Dioxolane demonstrates a low order of acute toxicity to mammals by the oral, inhalation and dermal routes. Genotoxicity has been evaluated using multiple in *vitro* and in *vivo* experimental procedures covering both mutation and chromosome aberration. The weight of evidence indicates lack of significant genotoxic properties. Reliable repeat-dose studies have been conducted by the oral and inhalation routes and a high-reliability 13-week inhalation study is available. The blood forming system was found to be the most sensitive target organ with a clear 13-week NOAEL of 300 ppm by inhalation. Kcproductive toxicity has been evaluated in one-generation drinking water and inhalation studies. Adverse reproductive effects are absent at dosage levels below maternally toxic doses. Developmental toxicity results are available indicating that Dioxolane is not a specific developmental toxin.

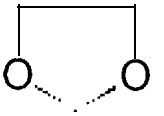
No additional testing is required under the EPA HPV Challenge Program as all relevant parameters are sufficiently characterized by the existing data and acceptable modeling. The aquatic stability and poor biodegradation in an OECD 301 ready biodegradation test indicate exposure of humans and aquatic organisms by way of the aqueous compartment would be the only area of potential concern. Data from the manufacturers, however, indicate that the level of Dioxolane being released from waster treatment plants is below the limit of detection or very low relative to its effect level. This suggests degradation in waste treatment plants and, coupled with the low toxicity, indicate low potential for harm by this route. Industrial exposures are two orders of magnitude below the subchronic NOEL and consumer exposures are negligible. There is a low priority for additional work on Dioxolane.

Test Plan in Table Format

CAS Number 646-06-o 1,3-Dioxolane	Information	OECD Study	GLP Study	Other Study	Estimation	Acceptable	Testing Required	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	N	N	Y	N	
Partition Coefficient	Y	N	N	N	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	Y	Y	N	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	Y	Y	N	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	Y	Y	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	Y	Y	N	Y	N	
72-96-Hour Algae	Y	Y	Y	Y	N	Y	N	
Toxicity								
Acute Oral	Y	N	N	Y	N	Y	N	
Acute Inhal	Y	N	N	Y	N	Y	N	
Acute Dermal	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	Y	Y	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology *in vitro*	Y	N	N	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N	

Introduction

I ,3-Dioxolane, CAS Number 646-06-0, (Dioxolane) is a cyclic reaction product of ethylene glycol and formaldehyde. It is a volatile liquid miscible with water in all proportions. Its structure is:



Use Pattern

The primary uses for Dioxolane are:

- Co-monomer for manufacture of polyacetals and other polymers.
- Solvent for chemical reactions (including inorganic salts).
- Stabilizer for halogenated organic solvents
- As a starting material or reagent for organic synthesis

Among these potential uses 95% or tnore of the current production is consumed in either the production of polyacetals or as a starting material for the production of a drug substance. Almost all of the remainder is used as a process solvent. There arc currently no consumer applications for Dioxolane. Thus, all exposure occurs in industrial settings where the material is a desired reaction product or used as a reactant or solvent. In these circumstances, exposures are well coutrolled by standard industrial hygiene practices. The majority end-products of Dioxolane, Polyacetals, are very high molecular weight compounds of little toxicological concern. This plastic is strong and ridged and replaces metals in many engineering applications, especially where low-friction properties are important. The polymer contains a very low level of free unpolymerized Dioxolane and consumer exposure represents a negligible risk. FDA has approved polyacetal for food contact USC and NSF has approved it for potable water.

Exposure Information

The limited use pattern for Dioxolane reduces the potential for exposure of humans or environmental species. Human exposure is thought to be limited to production workers involved in the manufacture of Dioxolane, the production ofpolyacetals, or the use of Dioxolane as a chemical intermediate. Exposure data from industrial hygiene monitoring of production and polyacetal manufacturing areas from a major producer indicates that worker exposure levels are low. In a period of several years of monitoring, data from 91 separate measurements of air concentration (time-weighted averages of 6 to 12 hour monitoring) showed an average level of 0.29 ± 0.39 ppm. The range of values was from 0 to 1.6 ppm. Another producer conducts annual

monitoring and the value has been less than 1 ppm (the level of sensitivity) over the lifetime of the monitoring program.

Environmental releases of Dioxolane could occur from released vapors or from wastewater effluent. Measurements of released vapor concentration arc not available, but vapor release is considered minimal based on levels measured in the workplace. Wastewater has been monitored for releases and Dioxolane concentration has been found to be below the limit of detection (0.1 ppm) in one plant. In another plant, it was found to average about 4 ppm with a higher release just after startup of production and Dioxolane release falling to below the limit of detection (1 ppm) after the start-up period. All known users of commercial Dioxolanc have an approved wastewater treatment facility on site. Thus, aquatic releases are considered minimal.

Synonyms

- CASRN: 646-06-o
- 1,3-Dioxacyclopentane
- 1,3-Dioxolan
- Dioxolane
- 1,3-Dioxole, dihydro-
- Ethylene glycol formal
- Formal glycol
- Glycolformal
- Glycol inethylene ether

Physical-chemical Data (HPV)

Physical-chemical data for Dioxolane are available from the literature and company sources.

Melting Point	**-95** deg C [1]
Boiling Point	**78 C @ 765** mm Hg [1]
Vapor Pressure	**70** mm at 20 deg C. [2] **79** mm at 20 deg C. [3]
Partition Coefficient	log $K_{o/w}$ = - 0.37 [4]
Water Solubility	Soluble in all proportions [1]

Summary: Dioxolane is a volatile water-soluble liquid that distributes preferentially into water over n-octanol.

Recommendation: Dioxolane is sufficiently well characterized regarding these parameters for the purposes of hazard and risk assessment. No additional work is recommended.

Miscellaneous Properties (non-HPV)

Empirical formula	$C_3H_6O_2$
Molecular Weight	74.09
Flash Point:	35 deg F (1.67 deg C) (Open Cup) [2]
Density/Specific Gravity:	1.0600 @ 20 deg C
Bulk Density:	8.2 lb/gal @ 20 deg C. [2]
Solubilities:	Soluble in water, alcohol, ether, acetone

Environmental Fate and Pathways

Photodegradation

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. [5] The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. In the case of Dioxolane, the APOWIN estimate for the reaction rate is based on simple hydrogen abstraction. Compounds similar to Dioxolane give a good correlation between estimates and measured values for this rate constant. Thus, this method is expected to provide an accurate estimate of the reaction rate constant with hydroxyl radical. Using this estimated value and the default in APOWIN for atmospheric hydroxyl radical concentration, the estimated half-life is approximately 11.5 hours.

Another estimate for atmospheric fate is reported in the National Library of Medicine's Hazardous Substance Data Base tile (HSDB) as follows. "Based on the vapor pressure of 79 mm Hg at 20 deg C, 1,3-dioxolane is expected to exist almost entirely in the vapor phase in the ambient atmosphere. Vapor phase 1,3-dioxolane is degraded in the ambient atmosphere by reaction with photochemically formed hydroxyl radicals; the half-life for this reaction in air can be estimated to be about 1. 1 days. [6]" The reference used in this HSDB entry is to the Atkinson SAK method for estimation of the rate constant for the reaction of various organic chemicals with hydroxyl radicals in the gas phase. This estimate varies only slightly from that obtained with the current APOWIN program in EPIWIN [5].

Water Stability

Water stability has been determined for Dioxolane using an OECD 111 compliant protocol. In this study, Dioxolane was incubated for four days at pH 4, 7 and 9 at 51 ° C. Hydrolysis was below five percent at all pH values. Accordingly, Dioxolane is estimated to be stable in the aquatic environment under typical environmental conditions for over one year. [7] It should be recognized that volatilization would occur and result in loss of Dioxolane if the aquatic system is exposed to air.

Theoretical Distribution

Theoretical Distribution (Fugacity) was estimated using the MacKay (Level III) model with standard defaults contained in EPIWIN v 3.05. The results for distribution using a model calculated $K_{o/c}$ of 0.175 are:

- o Air **4.1 %**
- 0 Water **54 %**
- 0 Soil **42 %**
- o Sediment 0.1 %

Biodegradation

A recent Closed-Bottle Test (OECD 301D) indicates that Dioxolane is not considered readily biodegradable, consuming only 3.7% of the theoretical oxygen demand after 35 days of incubation. Positive and negative controls in this study were in the normal range. [8]

A screening level BOD_{15} study sponsored by Celanese provides support for the closed bottle test. In this study using municipal secondary sludge, the BOD_{15} was 0.063 mg oxygen taken up per mg compound. This indicates a 12% biodegradation in 15 days for dioxolane. The BOD_{10} was reported to be 0.035 mg suggesting that moderate to slow degradation was occurring under these conditions.'

Volatilization from surface waters is expected to be a significant means of loss from the aquatic compartment. This estimate is based on the experimentally derived Henry's Law Constant of $2.4x10^{-5}$ atm-cu m/mole at 25°C.[10] The experimental Henry's Law Constant is also given in the EPI WIN program as $2.45x10^{-5}$ atm-cu m/mole and referenced as Cabani et al. [11] Both of these experimental values arc in accord with the estimate of $2.23x10^{-5}$ atm-cu m/mole calculated by the HENRYWIN v3.1 program found in EPIWIN. [5] The extent of biotic and abiotic degradation has been established to be low since Dioxolane is stable in water and does not readily biodegrade.

The log $K_{o/w}$ of -0.37 suggests low bioaccumulation potential.

Summary: Dioxolane is chemically stable in the aquatic environment. It does not readily biodegrade and the primary fate for material entering the environment is photodegradation by atmospheric hydroxyl radical. The high volatility and magnitude of the Henry's Law constant suggest that Dioxolane will efficiently volatilize from open systems and undergo atmospheric photo-oxidation. The Octanol-water partition coefficient indicates that it has low potential for bioaccumulation.

Recommendation: Physical constants necessary for estimation of distribution of Dioxolane in the environment are well known and no additional work is recommended for physical constants. Regarding fate in the environment, no new testing is required under HPV. Although Dioxolane is

hydrolytically stable and not readily biodegradable, the manufacturers find that the Dioxolane is either undetectable (limit of detection 0.1 ppm) in waste-treatment plants or reduced by about 85% by treatment [12 13]. These data suggest that Dioxolane is to a great extent removed by an acclimated wastewater treatment facility and, since it has demonstrated low toxicity to aquatic organisms, no additional testing is recommended.

Ecotoxicity

Fish

Dioxolane was found to have a low order of toxicity to typical aquatic environmental species. The LC_{50} (96 -hour) for freshwater fish was found to be greater than 95.4 mg/l in a recent OECD 203 guideline compliant study. [14] The LC_0 and the NOEC were found to be 95.4 mg/l. The study was conducted as a static-renewal study with daily renewal of test solution to prevent loss due to volatilization. No mortality was observed in this limit test.

A screening level study in saltwater fish, sponsored by Celanese, was conducted in which Shecpshead minnows (*Cyprinodon variegates, five* per group) were exposed to Dioxolane at concentrations of 7500, 11000, l 3000, 15000 and 25000 mg/l. In this study, the 48-hour LC_{50} was reported to be 12000 mg/l and the 96-hour LC_0 was 7500 mg/l, the 96-hour LC_{50} was reported to be 10000 mg/l. A clear dose-response was established with a 24-hour mortality of 5/5 at 25000 mg/l.[9] Based on the volatility of Dioxolane, the actual value for the EC_{50} in this study is likely lower than reported since the actual average concentration was probably lower than the nominal concentration. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward bluegill sunfish in the OECD 203 guideline study. Other support for a low-level of toxicity comes from modeling. Using the EPA ECOSAR model found in EPIWIN, the 96-hour estimated LC_{50} for fish is 8150 mg/l, a value close to that reported in the screening-level study. [5]

Aquatic Plants

Adverse effects on aquatic plants were examined in two studies. The first is a recent OECD 201 compliant study in which the E_bC_{50} and E_rC_{50} (O-72 hours) were >877 mg /l (based on measured concentrations). The 72-hour no-observable-effect concentration (NOEC) was 877 mg/l. [15] The 877 mg/l concentration was the highest level tested. The second study is an older screening level study in which Dioxolane was tested for growth inhibition of *Selenastrum capricornutum* In this study, algae growth was measured out to 14 days past initial exposure at levels of 1000, 5000 or 10000 mg/l with counts recorded on day 3 and later. Significant inhibition was seen only at 5000 mg/l and above. In this study, 1000 mg/l was determined to be the NOEC. [9] The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour EC_{50} for green algae to be 4075 mg/l.[5]

Aquatic Invertebrates

Daphnia toxicity was evaluated in a definitive OECD guideline study and in a screening-level study. In the first, which followed the OECD 202 guideline, the EC_{50} (48 hour) was >772 mg/l

under static renewal conditions to ensure exposure to representative concentrations of Dioxolane.'"

The second study is a screening level study, in which Dioxolane was tested at 1000, 5000, 6500, 8000, 9000, 10000 or 12500 mg/l. In this study, the 24-hour EC_{50} was reported to be 7650 mg/l and the 48-hour EC_{50} was reported to be 6950 mg/l. Twenty daphnids were exposed and a clear dose-response relationship was obtained. [9] Based on the volatility of Dioxolane, the true value for the EC_{50} in this study may be lower than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward daphnids. Using the EPA ECOSAR model, found in EPIWIN, the 48-hour estimated LC_{50} for daphnia is 7,400 mg/l. [5]

Summary: The ecotoxicity results clearly indicate that Dioxolane is of low concern to aquatic environmental species. In addition, the volatility of Dioxolane would limit the level of material found in surface water.

Recommendation: The toxicity of Dioxolane to aquatic organisms is sufficiently well established. The high volatility limits the extent to which terrestrial organisms will be exposed to Dioxolane. No additional testing is required.

Health Elements

Acute Toxicity

Oral Route

The oral LD_{50} in the rat was determined to be 5,200 mg/kg in a 1980 study sponsored by Celanese Corporation. [17] This was a tivc-dose study and the clinical signs associated with gavage exposure arc well documented. In another study found in the literature, Czajkowska, et al. [18] reported the oral LD_{50} of Dioxolane as 5,800 mg/kg. A limit test conducted by Hoechst [19], reported the oral LD_{50} to be greater than 2000 mg/kg. In a 1949 report, Smyth, Carpenter and Weil reported the oral LD_{50} of Dioxolane as 3000 mg/kg. [20]

Dermal Route

In a journal article by Czajkowska et al., the dermal LD_{50} of Dioxolane was reported to be about 15,000 mg/kg in albino rabbits. [18]

Inhalation Route

Acute inhalation studies revealed that the 4-hour acute LC_{50} is 68.4 mg/l in Sprague-Dawley rats. Nominal exposure concentrations for were 201.9, 88.4, 67.9, 60.6 and 37.9, milligrams per liter

(mg/l). Respiratory and neuromuscular abnormalities were the immediate responses to the test material during the exposure and during the four hour-post-exposure observation periods. Severity and incidences of these findings followed a concentration-related pattern. During the 14-day post-exposure observation period, all survivors appeared to recover by day 4. Most surviving animals appeared to regain pre-exposure body weights by Day 7 and showed normal weight gain patterns during the second week. Necropsy findings revealed high incidences of lung and liver discoloration as well as bladders distended with fluid and gastrointestinal tracts distended with gas in animals dying prior to scheduled sacrifice. The frequency of these findings appeared to be concentration related. [21]

Czajkowska, et al, also reported acute inhalation study results. [18] The LC_{50} concentrations were 118 mg/l in the rabbit (exposure time not given), 87 mg/l/ (4 hours) in the male rat and 166 mg/l (4 hours) in the guinea pig.

Summary: The acute toxicity of Dioxolane is well characterized by the available studies. There is a good correlation between the oral LD_{50} and the inhalation LD_{50}. Using a typical value of 75 ml for the minute volume of a 250-gram rat, the inhalation LD_{50} corresponds to a rat breathing in about 4900 mg/kg during the 4-hour inhalation exposure; this is similar to the oral dose producing lethality. Deaths occurred rapidly suggesting a CNS/respiratory system depression as the likely mechanism. Surviving animals showed normal weight gains within a few days after exposure.

Recommendation: Acute toxicity is sufficiently well characterized by the available studies. No additional testing is recommended.

Repeated Exposure Toxicity

Oral

In a 14-day gavage study conducted by Hoechst Celanese Corporation [22], groups of 10 rats of each sex were administered Dioxolane in corn oil by gavage for 14 consecutive days. Dosage levels were 0, 75, 250,750 and 2000 mg/kg/day, plus a water-gavage control. Animals were sacrificed on the 15"' day and necropsied. I Iistopathologic examination was conducted on several organs. Blood was removed for hematology. Chemically related mortality was limited to three high-dose males and four high-dose fetnalcs during the study. Male rats at dosages of 250,750 and 2000 mg/kg/day showed dosage-dependent decreases in platelet counts and reduced body weight gains and feed consumption values. The 750 and 2000 mg/kg/day dose groups had increased relative weights of the liver and lungs and reduced relative weight of the thymus. The 2000 mg/kg/day dosage group had increased relative kidney weights and reduced relative spleen weight. The 2000 mg/kg/day dosage of Dioxolane also produced adverse clinical signs, necropsy findings and histopathologic changes in the liver, thymus, kidneys and testes (ccntrilobular hepatocellular hypertrophy and midzonal hepatocellular vacuolation; thymic atrophy; renal cortical tubular basophilia and dilatation and accumulations of birefringent intratubular crystals; subacute renal pyclitis and multifocal testicular degeneration (testicular degeneration in two of ten males).

In female rats, administration of Dioxolane at dosages of 750 and 2000 mg/kg/day caused increases in relative liver weights and a decrease in relative weights of spleen and thymus. The 2000 mg/kg/day dosage group also had increased relative kidney weights and a reduced relative pancreas weight. Dosages of 250 mg/kg/day and higher reduced body weight gains, feed consumption values and lymphocyte counts. Dosages of.750 and 2000 mg/ kg/day caused clinical observations, and the 2000 mg/kg/day dosage also caused a gross lesion, reduced platelet count and histopathologic changes in the liver, kidneys and thymus (centrilobular hepatocellular hypertrophy and midzonal hepatocellular vacuolation; thymic atrophy; subacute renal pyelitis). The NOEL for Dioxolane was 75 mg/kg/day.

In a 4-week "pilot study", groups of 20 male and 20 female albino rats, albino mice and Syrian hamsters were exposed to Dioxolane in drinking water at concentrations of 0, 0.5, I .0 and 2.0%. [23] Body weight and water consumption were the only parameters recorded. Rats of each sex showed reduced body weight gains at the two highest dose levels. Body weight gains of high-dose mice and hamsters also appear to be affected but did not achieve statistical significance. The authors of the report only noted the statistically significant changes in body weight gains for rats (males, 1 .O% and 2.0 % dose levels: females 2.0% dose level). Hamsters appear to be less sensitive to the toxic effects of dioxolane than rats and mice; rats appear to be the most sensitive based solely on body weight gains. The actual dosing for the male rats at 1% in drinking water with water consumption at 30 ml per day calculates to approximately 1000 mg/kg/day Dioxolane. This is the approximate dose level were body weight changes are observed in other repeat-dose studies.

Inhalation

A two week inhalation toxicity study, sponsored by Celanese was performed using groups of rats (5/sex/group) exposed for six hours per day, five days per week, for two weeks to Dioxolane vapor at concentrations of 0, 3 and IO mg/l (984 and 3280 ppm). All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals may have indicated a response to treatment.[24]

In a 2-week inhalation study sponsored by Dow Chemical Co, groups of five male and five female Fischer 344 rats were exposed to concentrations of Dioxolane targeted to be 0, 500, 2000, and 5000 ppm. Animals were exposed for 6 hours per day, five days/week for nine exposures at measured concentrations of 0, 5 16, 23 19, and 5 132 ppm. Study parameters included body weights, organ weights, hematology, clinical chemistry, and gross and microscopic pathology. Exposure related and concentration-dependent effects were slight incoordination (5000 ppm males and females) during and following each exposure, decreased mean body weight during week two of exposure (5000 ppm males and females), and decreased white blood cell (WBC) counts (2000 and 5000 ppm males and females). Transitory incoordination was not associated with microscopic changes in tissues examined from the central and peripheral nervous system. Decreased WBC counts were not associated with morphologic alterations in the bone marrow,

spleen, thymus, or lymph nodes. In addition, there were no exposure-related microscopic changes in any other organ or tissue. The NOEL for male and female rats was 500 ppm. [25]

Summary: The repeated-dose toxicity studies indicate that Dioxolane has a low order of toxicity with the most sensitive organ system probably being the blood-forming organs. There is a good correlation of effects and dose levels between lhe gavage and the inhalation studies. The NOAEL and LOAL for gavage were 75 and 250 mg/kg/day, respectively; and the NOAEL and LOAL of the 14-Day Dow inhalation studies were 500 and 2000 ppm, respectively. Using a 7.5 ml minute volume for a 250-gram rat and assuming 50% absorption, the 500 and 2000-ppm concentrations correspond to about 80 and 325 mg/kg/day absorbed Dioxolaue, respectively. Toxic effects and relative sensitivities, thus, appear not to be greatly route dependent.

Recommendation: The effects of repeated-dose administration of Dioxolane are sufficiently well characterized. No new studies are required

Subchronic Toxicity

A I i-week inhalation study in rats conducted by Dow Chemical Company (1990) used 10 rats per group and satellite groups of 10 rats of each sex per dose level and control that were allowed an g-week recovery period. Dose levels were 0,300, 1000 and 3000 ppm with a six-hour per day exposure five days a week. At the end of the exposure period, urine was collected, animals were sacrificed, blood was taken for hematology and chemical chemistry, and animals were submitted to a complete uecropsy. Several organs were weighed and many tissues were fixed, sectioned, stained and examined microscopically. The satellite animals had blood samples taken at four and thirteen weeks of exposure and again at four and eight weeks into the recovery period. Satellite rats were uecropsied at the end of the eight-week recovery period. In males, no significant effects were reported in the 300 or 1000 ppm group and the 3000 ppm group showed decreased alertness at the end of each exposure, reduction in leukocyte count, decreased urine specific gravity, decreased spleen weights and microscopic examination revealed that hepatocytes in the ccntrilobular regions of lobules were slightly larger and had more cytoplasmic eosinophilia than controls. In females, no significant effects were reported in the 300-ppm group. At 1000 ppm the females showed reduced spleen weights and increased relative liver weights. The 3000-ppm group showed decreased alertuess at the end of each exposure, reduction in leukocyte count, decreased urine specific gravity and decreased spleen weights. Overall, the primary effect of dioxolaue inhalation exposure to rats appears to be a reduction in white blood cells counts at 1000 or 3000 ppm. Rats appeared healthy, body weight gain was not affected and there was no corresponding myeloid toxicity, although there was a slight reduction in myeloid cells of the bone marrow at 3000 ppm. Exposure related pathologic effects **were** limited to slight enlargement of ccntrilobular hepatocytes of males at 3000 ppm. There was no indication of testicular toxicity based on careful light microscopic examination. The NOAEL was 1000 ppm for males and 300 ppm for females.[26]

Czajkowska et al. reported a seven-month gavage study. In this study, dioxolane was administered as a 20% aqueous solution to groups of 8-10 rats by gavage at 1/10 (580 mg/kg), 1/40 (145 mg/kg) and 1/80 (72.5 mg/kg) of the single-dose LD_{50} for 7 months. In addition, undiluted dioxolane was administered at 580 mg/kg to a group of rats for a period of 4 months. Mortality, appearance and behavior were recorded and body weight was determined once a month. After completion of the 7-month exposure period, some hematology and biochemistry parameters were measured, animals were necropsied and selected organ weights were determined. Effects were seen primarily in high-dose animals dosed with undiluted Dioxolane. These animals, dosed for only four months, showed reduced (about 20%) body weight gain, hypokinesis, slightly weakened muscular force and slight paresis of hind legs. The animals receiving the same dose level of Dioxolane as a water solution for seven months showed a slight increase in body weight gain. These high dose animals given aqueous dioxolane showed slightly reduced (20% as compared to control) activity of whole blood acetylcholinesterase. The 0.145 mg/kg dose group showed a 12% increase in activity of whole blood acetylcholinesterase. It was concluded that there was no evidence for "accumulative effects" of dioxolane under these conditions." It is not known why the effects of undiluted dioxolane were different from diluted dioxolane at the same dose on a mg/kg basis. The aqueous high-dose of 580 mg/kg/day appears to be a NOEL with regard to body weight gain which was the best-documented adverse effect.

Summary: Subchronic administration of Dioxolane produced effects similar to those reported in the repeated-dose studies. The most sensitive target organ in the subchronic inhalation study was the blood forming system, manifest as reduction in WBCs and platelets and changes in spleen weight appear in rats. The inhalation NOEL for these effects was found to be 300 ppm for females and 1000 ppm for males in a 13-week study. The seven-month gavage study indicated low toxicity for the test material. The potential for ncurotoxicity, suggested by the perceived neuromuscular effects at the high-dose level, was investigated by measurement of blood cholinesterase activity at the end of the exposure period. The statistically significant reduction in cholinesterase at the high dose was not supported by the lower dose, which showed higher activity than control. In addition, the correlation between blood cholinesterase and acetylcholinesterase in the neuronal receptor is weak and the magnitude of the observed depression is not convincing, especially in light of the length of exposure. [28] No other indication of neurotoxicity, other than transient solvent depression of the CNS, has been reported for Dioxolane and there is insufficient evidence to suggest neurotoxic potential based on these data.

Recommendation: The subchronic effects of Dioxolane are sufficiently well characterized for purposes of hazard and risk assessment. No new studies are recommended.

Genetic Toxicology In *Vitro*

The mutagenicity of Dioxolane was evaluated in Salmonella tester strains TA98, TA1 00, TA 1535, TA1537 and TA1538 (Ames Test), both in the presence and absence of added metabolic activation, using Aroclor-induced rat liver S9 fraction, in a study sponsored by Celanese and conducted by Litton Bionetics. Dose levels were selected based on a preliminary toxicity determination. Dioxolane, diluted in ethanol, was tested at concentrations up to 50 µl/plate using the plate incorporation technique. Dioxolane did not cause a positive response in any tester strain with or without metabolic activation. [29]

An Ames test of Dioxolane was conducted by Goodyear in Salmonella tester strains TA98, TA 100, TA 1535, TA1537 and TA1538, both in the presence and absence of added metabolic activation by Aroclor-induced rat liver S9 fraction. Dioxolane, diluted in DMSO, was tested at concentrations up to 1000 µg/plate using the plate incorporation technique. Dioxolane did not produce a reproducible positive response in any tester strain with or without metabolic activation."'

Another Ames test of Dioxolane was conducted at Hill Top Labs using Sahnonella tester strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of added metabolic activation by Aroclor-induced rat-liver S9 fraction. Dioxolane was tested using the plate incorporation technique at concentrations of 0.05 to 50 microliters per plate. Dioxolane did not demonstrate a positive mutagenic response under these conditions. [31]

The mutagenicity of Dioxolane was evaluated in *Salmonella* tester strains TA1535, TA1537 and TA 1538 (Ames Test), and in *Saccharomyces cerevisiae* strain D4, both in the presence and absence of added metabolic activation by induced rat, mouse or monkey tissue S9 fraction from liver, lung and testis from animals pretreated with a "mixture of polychlorinated biphenyls". Salmonella were incubated with test substance both as suspension incorporation tests (at 0.75 and 1 .5%) and as a plate incorporation test at 1.5% using rat liver lung and testis. With the tissues from other species, only plate-incorporation techniques were used for bacteria. Suspension tests with yeast were conducted at concentrations up to 5.0% using non-activation and activation conditions. Activation conditions for yeast cultures used only rat tissues (livers, lungs and testes). Dioxolane did not cause a positive response in any tester strain with or without metabolic activation. [32]

The ability of Dioxolane to cause aberrations in Chinese hamster ovary (CHO) cells in vitro was evaluated in the presence and absence of Aroclor-induced rat-liver S9 metabolic activation. Based on preliminary toxicity tests, nonactivated and activated cultures were treated with 0, 2.0, 3.0, 4.0 or 5.0 mg/ml. Nonactivated cultures were incubated with Dioxolane for 7.5 hrs and activated cultures were incubated for 2 hrs. A total of 100 cells were scored/each of two replicate cultures/dose level. There was no toxicity observed at the highest dose level in either the nonactivatcd or activated cultures. None of the test cultures exhibited a significant increase in the

frequency of cells with chromosome aberrations in either the nonactivated or activated systems compared to the negative control."

The ability of Dioxolane to induce specific locus mutations at the TK locus in cultured L5 178Y mouse lymphoma cells (Mouse Lymphoma Mutagenicity Assay) was evaluated in the presence and absence of Aroclor-induced rat liver S9 metabolic activation. Based on preliminary toxicity determinations, nonactivated cultures were tested with concentrations ranging from 750 nl/ml to 5000 nl/ml, producing a range of 8.5 - 61% relative growth. Activated cultures treated with concentrations ranging from 750nl/ml to 5000nl/ml produced a range of 77.8 - 95.1% relative growth. None of the cultures produced mutant frequencies significantly greater than the water control.[34]

Dioxolane was tested in the presence and absence of an activation system for detection of its DNA damaging potential in the SOS assay. This assay is based on the test agent inducing beta-galactosidase (GAL) activity in Salmonella-typhimurium strain (TA-1535/pSK1002). GAL is coded by the lacZ gene and controlled by the fused umuC and lacZ gene in the pSK plasmid.by induction of umu gene expression. The potential to induce SOS response was monitored by measuring the cellular 6-galactosidase activity. Under the conditions of this assay, Dioxolane was negative.[35]

Dioxolane was tested in C3H/101 1/2 cell cultures in vitro for cytotoxicity and cell transformation. Evidence of transformation was observed in the colony and foci assay methods. Because of the dose range employed and the type of transformation observed, Dioxolane was considered to have weak positive activity in C3H/101 1/2 cell cultures.[36] The data suggest transforming activity but there are serious issues of concern with the data. The poor reproducibility on different days, the lack of clear dose-concentration relationships, the test material sporadically giving results similar to positive controls and at other times no response, and the lack of high-concentration toxicity data makes it difficult to conclude that this assay represents a positive finding. A more appropriate interpretation of the data is "not interpretable" as to genotoxic potential.

Dioxolane was tested for its ability to produce cell transformation in Balb/c-3T3 cells. Treatments were conducted at multiple dose levels, including slightly cytotoxic concentrations, in sealed flasks to prevent volatilization. . The transforming activities of all five doses of Dioxolane were comparable to the spontaneous background average of 0.12 foci/culture vessel and there was no evidence of a dose-related response with the test material. This range of test material treatments corresponded to 0 to 85% survival in the simultaneous colony survival assay and did not induce a significant increase in the appearance of transformed foci. Therefore, the test material was considered to be inactive in the Balb/c-3T3 in vitro transformation assay.[37]

Genetic Toxicology *In Vivo*

The possible genotoxic effects of dioxotane were studied usiug the micronucleus test in a published study. Mice were given dioxotaue ip in two doses at 24 hr intervals. Dioxolaue at doses from 1500 mg/kg to 6000 mg/kg caused a significant iucrease of micronucleated polychromatic erythrocytes in bone marrow of mice as compared to the negative coutrot value. Positive results in the micronucleus test provide evidence of genotoxic activity for Dioxolane. This study was considered potentially spurious, as no other genotoxic activity of Dioxolane was known, the study was not conducted under gtp conditions, experimental details were lacking and the purity of the test material was not specified. [38] Because of the questions surrounding the reliability of this report, Hoechst Cetauese conducted a more robust gtp-study in which no genotoxic activity was found. This is reported below.

A gtp mouse micronucleus test was sponsored by Hoechst Celanese Corporation in which groups of five ICR mice of each sex received Dioxolanc in corn oil by i.p. iujectiou at 0, 1576, 2048, 2663, 3462 or 4500 mg/kq. Mortality at the end of a seven-day observation period was used to calculate an i.p. LD_{50} of 2603 mg/kg. The high dose for the micronucleus study was set at 80% of the LD_{50}, which was 2100 mg/kg. Lower levels were 1050 and 525 mg/kg. Groups of mice of each sex were injected with these three dose levels of Dioxolane and sacrificed in groups of five at 24, 48 and 72 hours post injection. Excessive mortality occurred at the high dose level and additiouat animals were injected to allow sufficient high-dose animals for sacrifice at 72 hours post iujectiou. The number of micronucleated cells was not increased by treatment with Dioxolaue while the number in the positive control was significantly iucreased and within the expected range. The high mortality observed in the high-dose-group and significant boue marrow toxicity at the high dose level iudicatcd that the test material was administered at the highest practical level. Under the conditions of this assay, Dioxolane did not show genotoxic activity. [39]

Dioxolane was tested for induction of single-strand breaks of DNA in rat hepatocytes in vivo. Male rats were injected iutraperitoneally 4 hours before decapitation with dioxotane at a single dose of290 mg/kg or 580 mg/kg. Single-strand breaks in DNA were detected by the alkaline elutiou technique. The elution rates were significantly higher than these of DNA from control rat hepatocytes, but no dose effect relationship was found. Under the conditions of this assay, dioxotane was considered to induce single-strand breaks in DNA. [40] These results are possibly spurious and related to an impurity of the test material which may have been derived from the same source as the test material used in the reported-positive micronucleus test.

Au oral evaluation of the ability of Dioxolane to increase the frequency of dominant lethal mutations in the germ cells of male rats was conducted. Male rats that received Dioxolane daily for eight weeks were evaluated for effects on fertility in a weekly mating design. Oral doses of 0, 580 and 1160 mg/kg/day were administered five days per week for eight weeks. Every week during the treatment period, each male was mated with two females. This dosing regime had no effect on mate fertility indexes. Pregnancy outcomes were also comparable to controls. System toxicity was apparent as body weight gains in thc 580 mg/kg dose tcvct were 47% of control and

body weight gain in the 1160 mg/kg dose group was only 22% of control. Liver kidney and spermatic vesicle weights were increased in dosed animals. Although no functional deficit in fertility was recognized in these males, microscopic evidence of testicular degeneration and alterations in spermatogenesis were noted. Three of nine high dose males showed atrophy, necrosis and seminiferous epithelium exfoliation and three low-dose males were reported to show seminiferous epithelium exfoliation (one animal showed the presence of large multinucleated cells). There was no evidence of a dominant lethal effect. [41]

A 12-month inhalation study was conducted using male rats to evaluate the ability of Dioxolane to increase the frequency of dominant lethal mutations in germ cells. Male rats were exposed to Dioxolane five hours daily, five days a week, for twelve months, at a concentration of 2500 mg/m3. At the end of the 12-month exposure period, males (14 per group) were mated through one week with female rats in a ratio of 1:2. Dams were sacrificed 13-14 days after the middle of mating intervals for evaluation of conception products. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to the appropriate control group. Dioxolane did not affect the fertility of males; however, histopathological changes in the testis were reported. The authors reported that the frequency of necrosis in the seminiferous epithelium was much higher in treated animals than in controls but details were not provided. No compound induced dominant lethal mutations in germ cells were Found to result from inhalation exposure to Dioxolane under these conditions .[42]

Summary: Dioxolane has been extensively tested in genotoxicity assays. The weight of the evidence clearly indicates lack of significant genotoxic potential for this material. In addition, the few studies which suggest genotoxicity carry lower reliabilities.

Recommendation: Dioxolane has been sufficiently characterized for genotoxic activity. **No** new studies are recommended.

Reproductive Toxicology

In a one-generation reproduction study, male Charles Kiver rats (5/group) were exposed to Dioxolane in their drinking water at concentrations of 0, 0.5 or 1.0% for 90 days prior to mating with previously untreated females (IO/group, two females per male). Treatment continued through the mating period. Dams and pups were examined with respect to survival and body weight through postpartum day (PD) 2 l and the animals were retained for further experimentation. Significant differences were observed between treated and control animals in the following: decreased maternal body weights (high-dose group on PD 1 and 4, decreased rates of coupling and parturition, and increased number of stillborn pups (both treatment levels), decreased survival of pups in both treatment groups, and decreased numbers of pups in the high-dose group. No significant differences were observed between treated and control animals in the following: parental mortality, fecundity or female fertility indices, gross external abnormalities, and pup body weights. [43] In the second phase of the study, untreated proven-breeder male rats were mated with the dams that produced the F1a litter. The dams were treated with test material

continually from the initial (Fla) mating throughout gestation, lactation and a 10-day rest period after weaning of F la litter before the second mating period. Neither dams nor males were exposed after the Flb mating interval started. Dams and F1 b pups were examined with respect to survival and body weight through postpartum day 2 1, the Flb pups were retained for further testing, and the dams were sacrificed and subjected to histopathological examination. Significant differences observed between treated and control animals in the following: decreased fecundity index, incidence of parturition, and female fertility index at both treatment levels. No significant differences were observed between treated and control animals in parental mortality, male fertility index, number of pups born, stillborn, cannibalized, or viable pups (at birth or on lactation days 1, 4, 12 and 21), maternal or pup body weights, gross maternal pathologic examination, weights maternal organs (adrenals, both gonads, liver, pituitary, and uterus), and maternal histopathologic examination. [44]

In a one-generation reproduction study, male Charles River rats (5/group) were orally exposed to dioxolane in their drinking water at concentrations of 0, 0.01, 0.03 or 0.10% for 90 days prior to mating with previously untreated females (IO/group, 2 females/male). Treatment continued through mating, gestation and lactation. Dams and pups were examined with respect to survival and body weight through postpartum day (PD) 2 1 and the animals were retained for further experimentation. A significant, but likely spurious, difference was observed between treated and control animals in male pup body weight (decreased, low-dose group on lactation day 21). No significant differences were observed between treated and control animals in the following: maternal mortality, mating and fertility indices, male and female fertility, incidence of parturition, gross abnormalities in pups, number of pups delivered stillborn or viable, or cannibalized (lactation days 1, 4, 12 or 2 1), survival indices, female pup body weight, and female parental body weight. [45] The high dose, 0.10% in drinking water, is considered a LOAEL.

In a single-generation reproduction study, male Charles River COBS albino rats (5/group) were exposed by inhalation to Dioxolane at nominal (measured concentrations were close to nominal values) concentrations of 0 or 125 ppm for seven hours a day, five days a week, for 90 days prior to mating with untreated females (1 O/group, 2/male). Each sex was treated during mating and females were treated until l-2 days before delivery. Females and F la pups were retained for further studies, males were sacrificed and necropsied after mating. A significant difference was observed between treated and control animals with respect to decreased relative paternal lung weight for the Fla male parental generation. No significant differences were observed between treated and control animals in the following: mating, fecundity, male female fertility indices, incidence of parturition, gross external pup abnormalities, number of pups delivered, stillborn, cannibalized or viable pups and number of viable pups through 2 1 days, pup survival and body weights, maternal body weights, gross paternal necropsy examinations. [46] In the second part of this study, after weaning the F1 a litter, dams were mated with new males that had been exposed to Dioxolane for 120 days prior to mating. Rats of each sex were exposed to Dioxolane vapors during the 15-day mating period and the pregnant females were exposed until l-2 days before delivery. No significant diffcrcnccs were observed between treated and control animals in

fecundity or male fertility indices, ratio of parturition/pregnancy, gross external abnormalities in pups, number of pups delivered, delivered stillborn, cannibalized or viable, or number viable pups or mean pup weight through 2 1 days, F 1 b pups survival data, and maternal body weight. The female mating index was reduced but not with statistical significance. [47] In summary, although treatment did not significantly affect any measured reproductive parameter, there was a tendency toward an overall reduction in the number of pups born and weaned in both the Fla and Flb litters of the dosed group. The lack of statistical significance may partly be a function of the low numbers of animals employed in the study. The possibility that the 125-ppm exposure represents a LOAEL for reproduction in the Fla and Flb litters cannot be excluded.

Effects on male fertility and the frequency of dominant lethal mutation in germ cells of male rats were studies by exposing male rats to 2500 mg/m^3 (830 ppm) Dioxolane, five hours a day, five days a week for 12 months or by exposing male rats to oral gavage administration of Dioxolane for eight weeks at 580 or 1160 mg/kg/day (5 days per week for 8 weeks). Exposed rats were mated with unexposed females. Male fertility was unaffected by these dosing regimes as were other parameters indicative of a dominant lethal effect. Some treated males were reported to display focal necrosis of the seminiferous tubules. These studies are more fully described in the genotoxicity section of this document. [42]

Discussion of Reproductive Toxicology

Adverse reproductive effects of Dioxolane were reported at high dose levels in the drinking water reproduction (one-generation) studies. Most notable effects were the apparent reduced female mating index, live birth index and pup survival index at the high dose level (1 .0% in drinking water) for the F 1 a litter in which the females were exposed during mating and gestation. Examination of the data suggests that effects occurred at the 0.5% drinking water level and were more severe at the higher dose. The other drinking water study, at lower concentrations, was below the NOAEL. It could not be clearly determined which was the affected sex due to the study design. The LOAEL for reproductive effects may also be an effect level for maternal toxicity. There was clear maternal toxicity at the 1 .O% level and mild effects on body weight gain at the 0.5% level. This same strain of rats was also examined in a 4-week "pilot" study where only body-weight gains and water consumption was recorded. [23] In this study, body weight gain was reduced over the 4-weeks of the study for female rats by 15, 28 and 56 percent at 0.5, 1.0 and 2.0 percent drinking water levels, respectively. Although the reduced body weight gain was not statistically significant at the two lower doses (due to an n of only 5), it appears that 0.5% represents a maternally toxic concentration in drinking water.

Adverse effects on reproduction thus are considered to occur at maternally toxic doses. After consideration of the data and the information that higher gavage or inhalation doses do not cause dominant lethal effects and the additional data from the Sitarek et al study [49], it can be concluded that the affected sex in this study was probably the female. The perinatal mortality reported is supported by the developmental toxicity study of Sitarck el al. [49] (Described in detail under

developmental toxicology) in which increased perinatal mortality was reported after dosing pregnant dams every-other-day at 1150 mg/kg Dioxolane.

Another consideration in assessing the potential reproductive toxicity of Dioxolane is histopathologic changes in the reproductive organs. Three repeat-dose or subchronic glp-studies conducted careful examinations of male and female reproductive organs from exposed rats and in only one study was there any finding associated with reproductive organs. In the 14-day gavage study at doses up to 2000 mg/kg/day [22], two of ten high-dose males displayed multifocal testicular degeneration which was described by the pathologist as "the affected tubules had a decreased amount of spermatogenic cells with evidence of degeneration and formation of multinucleated giant cells" Other males at this dose-level were not affected or were females (regarding their reproductive organs) or any lower-dose animals. In the 14-day inhalation study at concentrations up to 5132 ppm [25] (inhalation of approximately 1700 mg/kg over 6 hours) no male or female demonstrated reproductive organ effects. And, in the 90-day inhalation study at concentrations up to 3010 ppm [26] (inhalation of approximately 1000 mg/kg over 6 hours), neither males nor females were found with adverse changes to reproductive organs. Taken together, this is strong evidence that Dioxolane is not a significant reproductive toxicant.

Summary: Dioxolane has an effect on reproductive parameters only at maternally toxic doses. The clear NOAEL for these effects was 0.1% in drinking water.

Recommendation: Reproductive effects of Dioxolane are characterized sufficiently for the purpose of hazard and risk assessment. No additional studies arc recommended.

Developmental Toxicology

In a developmental toxicity study sponsored by Hoechst Celanese in 199 1, groups of 25 presumed-pregnant rats received Dioxolane in corn oil by gavage from day 6 to 15 of gestation. Dosage levels were 0, 125,250, 500 and 1000 mg/kg/day. Animals were sacrificed on gestation-day 20, opened and examined for pregnancy, number and placement of implantation sites, early and late resorption, live and dead fetuses and number of corpora lutea. Fetuses were subsequently examined for gross external, soft tissue and skeletal alterations. No rats died during the study and adverse clinical signs were not reported at any dose level. Body weights of dams were reduced between gestation day (gd) six and seven for 500 and 1000 mg/kg animals. Transient reductions in feed consumption occurred for the 500 and 1000 mg/kg dose groups. Data from the preliminary range-finding study also indicate that dose levels of 250 mg/kg and above are associated with adverse effects related to the toxicity of the test material. The 1000 mg/kg dosage level was associated with significantly reduced fetal body weights and significantly increased litter and fetal incidences of externally evident tail malformations, vertebral malformations interrelated with the tail malformations and septal effects in the heart. High dose fetuses also displayed delayed ossification and one fetus was found with a cleft palate. Significant adverse fetal effects were not found in the 500-mg/kg group. The NOEL for dams was considered

to bc 250 mg/kg and the developmental NOEL was considered to be 500 mg/kg. Dioxolane, therefore, is not considered a specific developmental toxicant under the conditions of this study. [48]

A 1992 publication authored by Sitarek et al. [49] describes a developmental toxicity study of Dioxolane. The study was divided into two parts: prenatal and postnatal development. In the first part, female rats were given by gavage every other day from days 8-20 of gestation an aqueous solution of dioxolane at daily doses equal to 0.025, 0.1 and 0.2 LD_{50} (0.14, 0.58 or 1.15 g/kd/). In the second part (perinatal toxicity), Dioxolane was administered every other day from days 2-20 ofgcstation at daily doses equal to 0.025, 0.075 and 0.15 LD_{50} (as stated in the material and methods section, dose levels reported in the results and discussion section are the same as in the first experiment and it cannot be determined what doses were actually used in the second part). Dioxolane did not cause increased embryo or fetus intrauterine death rates or congenital defects at any dose level. In the pre-natal study, the mid (0.58 g/kg) and high dose (1.15 g/kg) were reported to bc associated with dose-related delays in fetal development. High-dose pups also weighed less and showed reduced mean crown-rump lengths. Examination of the presented data suggests that the mid-dose effect is marginal and probably represents a fetal NOEL of 0.58 g/kg. Maternal toxicity was manifest at the high dose by reduction in body weight gain and an increase in relative adrenal weights at sacrifice the maternal NOEL appears to bc 0.58 g/kg.

In the perinatal study, Dioxolane did not cause itnpairment of physical development or behavioral disturbances. Exposure to higher doses of the compound (stated as 1.15 g/kg) leads to increased pcrinatal death rates in the offspring, without causing disturbances in the maternal instinct. The exposure of pregnant rats to dioxolane decreased hemoglobin levels in 5-week-old offspring but the levels had returned to control by 8 weeks. At a dose 1.15 g/kg (0.2 LD_{50}) the chemical significantly increased exploratory tnotor activity of female offspring at the age of 8 weeks, but did not affect significantly locomotor activity of males and the active avoidance acquisition of adult offspring. High-dose males were reported to have reduced body-weight gain between the 14"' and 15"' week after birth. Perinatal deaths were also increased it the high-dose group.

These two developmental toxicity studies used an unusual (every other day) dosing schedule that might be expected to increase developmental toxicity relative to maternal toxicity. This makes it more difficult to compare these results to others. The lack of significant malformations, and the apparent equivalence of the maternal and the developmental NOELs suggest low potential for developmental toxicity from Dioxolane exposure.

Summary: the pre-natal developmental toxicity of Dioxolane had been well characterized by a 4-dose gavage study detnonstrating that Dioxolanc is not a specific developmental toxin. At maternally toxic doses, administration is associated with increased malformations and delayed ossification. The study by Sitarek is supporting in that doses of 575 or 1 150 mg/kg/day were not found to cause adverse effects on the conceptus other than devclopmental delays. lt is not known, however, why malformations were not reported at these maternally toxic doses since they were reported in the corn-oil gavagc (Argus) study. The dosing vehicle in the Argus study was corn oil

while the Sitarek study used water; differences in pharmacokinetics based on absorption rates are a possible explanation. Nevertheless, the developmental toxicity of Dioxolane appears low.

Recommendation: No new studies are recommended as this endpoint is sufficiently well characterized.

Other Studies (Non-HPV)

Absorption/Distribution/Metabolism/Excretion

Snipes et al. published a paper entitled *A method for measuring nasal and lung uptake of inhaled vapor* in which Dioxolane was used a model compound for demonstrating methodology. This paper describes apparatus and methods for measuring uptake of inhaled vapors in the nose and lungs of dogs. The system allows sampling of air from the trachea at specific times during inspiration and expiration without surgical manipulation of the animal, thus allowing repeated studies in the same animal. During exposure, the dogs are anesthetized and cyclic respiratory patterns arc maintained by means of an external respirator. A pneumotachograph installed in the exposure line is connected to a respiratory monitoring system that both monitors the dog's respiratory pattern and triggers sampling at specific times in the respiratory cycle. Air sampling, both at the nose and within the trachea, can be done during the entire breathing cycle or during specific portions of it. Vapors are sampled at a point just external to the dog's nose and from within the trachea through a modified endotracheal tube. To develop and demonstrate the system, three beagle dogs were exposed to Dioxolanc at nominal vapor concentrations of 500 ppm; vapor sampling was triggered for the entire inspiratory and expiratory portions of the breathing cycle during IO-min exposures. After correcting data to account for vapor that desorbed from the nasal passages during exhalation (after initially being absorbed in the nose during inhalation), net nasal uptake of Dioxolane was 66.6%. Lung uptake was 2.1% for Dioxolane. [50]

It can be concluded from this study that upper respiratory tract uptake of Dioxolane is an important absorption mechanism for dogs under these experimental conditions. Upper respiratory tract absorption is likely to also be an important uptake mechanism in humans.

Carcinogenicity

No known completed and reported studies of the carcinogenic potential of Dioxolane are available; however, the literature indicates that at least two had been started.

PPG Corporation sponsored a chronic (2-year) drinking water study at Industrial Bio-Test Laboratories. The in-life portion of the study was started in April 1975 and a draft report dated 7/29/1 98.5 was audited against laboratory data by Experimental Pathology Laboratories (EPL). In the August 1985 audit report from EPL, it was concluded that the study contains accurate toxicological information concerning the effects of dioxolane administered to the drinking water of male and female rats. The study had concluded that there were no statistically significant treatment-related effects in body weight or food and water consumption. A slight reduction in testicular weights and an increase in spleen weights in treated male rats was not statistically-

significant[51]. The final report of the chronic study was not found in the open literature and it is presumed that it was never issued.

There is reference to a chronic inhalation study in progress from which males were used as breeders for the one-generation inhalation reproduction study.46 It is not known if this chronic inhalation study was completed or reported.

Conclusions

Examination of the entire data set for Dioxolane indicates that there is a low priority for additional studies on this material. Dioxolane is mildly toxic by oral administration or by inhalation. After acute exposure to high levels, reversible CNS depression, typical of most volatile solvents, appears to be the primary effect. After repeated dose or subchronic administration, effects on the blood forming systems, manifest as reduction in WBCs and platelets and changes in spleen weight appear to be the most sensitive target organs in rats. The inhalation NOEL for these effects was found to be 300 ppm for females and 1000 ppm for males in a 13-week study. The oral gavage NOEL was found to be 75 mg/kg/day in a 14-day study. After oral administration to pregnant females, developmental delays and perinatal mortality are found only at maternally toxic doses. Reproductive effects are only apparent at maternally toxic levels. The weight of evidence indicates that Dioxolane has low genotoxic potential.

The current exposure pattern is restricted to industrial workers involved in the manufacture and subsequent use of Dioxolane. Monitoring data indicate that typical exposures are below 1 .0 ppm, which is at least two orders of magnitude below the inhalation 13-week NOEL. It is unlikely that Dioxolane will have any adverse effect at this exposure level. Ferro and Ticona are aware of no consumer exposure to Dioxolane.

Studies on aquatic organisms indicate a low order of acute toxicity for Dioxolane to fish, invertebrates or aquatic plants. Volatility limits the concern for chronic effects in the aquatic environment and data from plant effluents indicate that the levels being released from manufacture are less than 0.1 ppm in plant outfalls.

This analysis shows that Dioxolane has a low potential for harming the either human health or the environment and it is concluded that additional studies would not add significant information. No additional studies are recommended to till the requirements of the EPA HPV Challenge Program.

References

[1] Lide, D.R. (ed). CRC Han dbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 1991-1992, p. 3-218

[2] Sax, N.I. and R.J. Lewis, Sr. (eds.). Hawley's Condensed Chemical Dictionary. l lth ed. New York: Van Nostrand Reinhold Co. p 425 (1987) .

[3] Riddick JA et al: Organic Solvents 4th ed. NY: Wiley (1986)

[4] Hansch C, Leo AJ: Medchem Project Issue No. 26 Claremont, CA: Pomona College (1985)

[5] EPIWIN v 3.05 Syracuse Research Corporation, Syracuse, NY (April 2000).

[6] Atkinson R; Environ Toxicol Chem 7: 435-42 (1988)

[7] l ,3-Dioxolane CAS No. 646-06-o: Hydrolysis as a Function of pH. Toxikon Corporation, Laboratory Project ID 00J0009f, October 2000.

[8] l ,3-Dioxolane (CAS 646-06-o): Ready Biodegradability: Closed Bottle Test. Toxikon Corporation September 2000, submitted to Ticona and Ferro Corporations.

[9] Report to Celanese Chemical Company Inc. on Toxicology and Fatc of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.

[10] Hine J, Mookerjee PK; J Org Chem 40:292-8(197.5).

[11] Cabani S, Conti G, Lepori L, Thermodynamic Study On Aqueous Dilute Solutions Of Organic Compounds. Part 2 - Cyclic Ethers. Trans. Faraday. Soc. 67: 1943-50 (197 1).

[12] Communication from Ticona LLP production facility 12 June 2000.

[13] Communication from Ferro Corporation 25 October 2 2000.

[14] 1,3-Dioxolane CAS No. 646-06-o: Acute Toxicity to Bluegill, Lepomis macrochirus, Under Static-Renewal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009g, October 2000.

[15] 1,3-Dioxolane: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum* , Under Static Test Conditions. Toxikon Laboratories, Jupiter FL, Project ID 00J0009b, 27 September 2000, submitted to and sponsored by Ticona Corporation and Fcrro Corporation

[16] 1,3-Dioxolane CAS No. 646-06-o: Acute Toxicity to the Water Flea, *Daphnia magna*, Under Static-Rcncwal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009c October 2000

[17] Acute Oral Toxicity Study in Rats, Bio/dynamics Inc., Project No. 6004-79 (06/05/80), Sponsored by The Celanese Corporation, New York, NY.

[18] Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.

[19] 1,3-Dioxolan - Profung der akuten oralen Toxizitatan der Wistar Ratte. Hoechst AG Pharma Research Toxicology Report No. 87.1441, September 1987

[20] Smyth, H.F., Carpenter, C.P. and Weil, C.S.: Rangefinding toxicity data, list III. J. Ind. Hyg, Toxicol. 3 l :60-62 (1949).

[21] An acute inhalation toxicity study of C-121 [1,3Dioxolane] in the rat. Project No. 79-7304, Bio/dynamics Inc., July l 1, 1980.

[22] Repeated Dose Oral Toxicity Study of 1,3-Dioxolane Administered Via Gavage to Crl:CD (SD)BR Rats. Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044, Laboratory Project ID 508-002P.

[23] Four-week pilot study with Dioxolane in drinking water of albino rats, albino mice and golden Syrian hamsters. Industrial BIO-Test Laboratories, Inc., Project No. 8560-10579, July 12, 1977.

[24] A two week inhalation toxicity study of C-l 21 (1,3-Dioxolane) in the rat. Bio/Dynamics Inc Project No, **X0-7429,** June 22, 1981.

[25] 1,3-Dioxolane: A two-week vapor inhalation study in Fischer 344 rats. The Dow Chemical Company, Toxicology Research Laboratory, Health and Environmental Sciences, Laboratory Project Study ID K-O 10634-005, August 28, 1989.

[26] 1,3-Dioxolane: 13-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company **1990**

[27] Czajkowska. T. and Krysiak, B.: Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I 1. Accumulation of toxic cffccts. Med. Pr. 38, 244-249 (1987).

[28] Wills, JH. The Measurement and Significance of Changes in the Cholinesterasc Activities of Erythrocyte and Plasma in Man and Animals. Crit Rev Toxicology 1:153-202 (1972).

[29] Litton Bionetics Inc.; Mutagenicity Evaluation of 1,3-Dioxolane in the Ames Salmonella/Microsome Plate Test, Final Report, (1980), EPA Document No. 878213536, Fiche No. OTS0205850

[30] **Goodyear** Health, Safety and Environmental Laboratory; Mutagenicity Evaluation of 1,3-Dioxolane; (1979), EPA Document No. 40-8364009, Fiche No. OTS05 10987

[31] Hill Top Laboratories, Project 83-0212-2 1. Tbe Salmonella/Microsomal Assay for Bacterial Mutagenic Activity of 1,3-Dioxolane for Grant Chemical Division Ferro Corp. 10 March 1983.

[32] Litton Bionetics Inc.; Mutagenic Evaluation of 1,3-Dioxolane, (1975), EPA Document No. 40-8364007, Fiche No. OTS05 10985

[33] Litton Bionetics, Inc.; Mutagenicity Evaluation of 1,3-Dioxolane in an In Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells, Final Report. (1985), EPA Document No. 40-8564054, Fiche No. OTS05 11019

[34] Litton Bionetics Inc.; Mutagenicity Evaluation of 1,3-Dioxolane in the Mouse Lymphoma Forward Mutation Assay, Final Report, (1985), EPADocument No. 40-8564054, Fiche No. OTS05 1 10 19

[35] Nakamura, S., Oda, Y., Shimada, T., Oki, 1. and Sugimoto, K.: SOS-inducing activity of chemical carcinogens **and** mutagens in Salmonella typhimurium TA 153 5/pSK 1002: examination with 15 1 chemicals. Mutat. Res. 192, 239-246 (1987)

[36] An assay of cell transformation and cytotoxicity in C3H 10T 1/2 clonal cell line for the test chemical C- 12 1 [1,3-Dioxolane). Environmental Pathology Laboratory, University of Minesota, August 27, 1981

[37] Evaluation of 1,3-Dioxolane in thc in vitro transformation of Balb/c-3T3 cells assay. Litton Bionetics, Inc., Project No. 20992, January 1985.

[38] Przybojewska B et al; Toxicol Lett 2 1: 349-52 (1984)

[39] Micronucleus Cytogenetic Assay in Mice. Test article C- 12 1 [1,3-Dioxolane]. Microbiological Associates Inc., Study No. T9034.122, December 28, 1989

[40] Jaros-Kaminska, B., Baranski, B. and Interaction of trioxane and dioxolane in *vitro* and *in vivo*. Studia biophysica 1214 (1985)

[41] Baranski, B., Stetkiewicz, T., Czajkowska, T., Sitarek, K. and Szymczak, W. : Evaluation of mutagenic and gonadotoxic properties of trioxane and dioxolane. **Med.** Pr. 35, 245-255 (1984).

[42] Baranski, B., Stctkiewicz, T., Czajkowska, T., Sitarek, K. and Szymczak, W. : Evaluation of mutagenic and **gonadotoxic** properties of trioxane and dioxolane. Med. Pr. 35, 245-255 (1984).

[43] Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxalane in Drinking Water in Albino Rats, Results of the FO Generation. (1975), EPA Document No. 8782 13523, Fiche No. OTS0205848.

[44] Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxalane in Drinking Water in Albino Rats, Results of the FO Generation and Proven Breeder Males. (I 975), EPA Document No. **8782 13524,** Fiche No. OTS0205848

[45] Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxalane in Drinking Water in Albino Rats, Results of the FO Generation. (1975), EPA Document No. 8782 13525, Fiche No. OTS0205848

[46] Industrial BIO-TEST Laboratories, Inc.; Single Generation Reproduction **Study** With the Vapors of Dioxolane in Albino Rats, Results of the FO and Fla Generations, **Status** Report. (1976), EPA Document No. 878213527, Fiche No. OTS0205848

[47] Industrial BIO-TEST Laboratories, Inc.; Single Generation Reproduction Study With the Vapors of Dioxolane in Albino Rats, Results of the FO and Flb Generations, Status Report. (1976), EPA Document No. 8782 13528, Fiche No. OTS02005848

[48] 1,3-Dioxolane : Oral developmental toxicity study in Crl :CDR BR VAF/PlUSR presumed pregnant rats. Argus Research Laboratories, Inc., Protocol 508-002, May 9, 199 1

[49] Sitarek K, Baranski B, Berlinska B. The effect of maternal exposure to dioxolane on prenatal and postnatal development in rats. Pol J Occup Med Environ Health 5: 159-66 (1992).

[50] Snipes MB, Spoo JW, Brookins LK, Jones SE, Mauderly JL, Orwat TB, Stiver JH and Dahl, AR. A method for measuring nasal and lung uptake of inhaled vapor. Fundam Appl Toxic01 16:81-91 (1991).

[51] Two-year chronic toxicity study with dioxolane in drinking water of albino rats - retrospective data audit with cover letter dated 08 1685 EPA/OTS; Doc #40-8664052